

**DATA EVALUATION RECORD**  
**ACUTE LC<sub>50</sub> TEST WITH AN ESTUARINE/MARINE SHRIMP**  
**72-3(C), OPPTS 850.1035**

1. **CHEMICAL:** 2,4-D DMA, aminopyralid      PC Code No.: 030019 and 005100/0050209

2. **TEST MATERIAL:** GF-2633      Purity: 43.0% 2,4-D DMA + 8.43% aminopyralid

3. **CITATION:**

Author: Bergfield, A.  
Title: GF-2633: Acute Toxicity to the Mysid Shrimp, *Americamysis bahia*, Determined Under Static-Renewal Test Conditions

Study Completion Date: November 11, 2011

Laboratory: ABC Laboratories, Inc.  
7200 E. ABC Lane  
Columbia, MO 65202

Sponsor: Dow AgroSciences LLC  
9330 Zionsville Rd.  
Indianapolis, IN 46268

Laboratory Report ID: 66956

MRID No.: 48939503


DP Barcode: 289122

4. **REVIEWED BY:** Rebecca L. Bryan, Staff Scientist, CSS-Dynamac

**Signature:** 

**Date:** 12/18/15

5. **APPROVED BY:** John Marton, Ph.D., Environmental Scientist, CDM Smith, Inc.

**Signature:** 

**Date:** 05/23/16

6. **APPROVED BY:**

**Primary Reviewer:** Rebecca Lazarus, Ph.D.  
EPA/OPP/EFED/ERB1

**Signature:** 

**Date:** 06/3/2016

**Secondary Reviewer:** {.....}  
EPA/OPP/EFED/ERB

**Signature:**

**Date:**   
6/3/16

7. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shrimp. It is not intended to prescribe conditions to any external

party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

**STUDY PARAMETERS:**

<b>Test Species and Strain:</b>	Mysid shrimp ( <i>Americamysis bahia</i> )
<b>Age or Size of Test Organism:</b>	<24 hours old at test initiation
<b>Definitive Test Duration:</b>	96 hours
<b>Study Method:</b>	Static-renewal
<b>Type of Concentrations:</b>	mean-measured

**8. CONCLUSIONS:****Results Synopsis for a 96 hour LC<sub>50</sub>**GF-2633

LC<sub>50</sub>: 84.1 mg form/L                      95% C.I.: 69.1-102 mg form/L

2,4-D DMA

LC<sub>50</sub>: 36.1 mg ai/L                      95% C.I.: 29.7-43.9 mg ai/L

Aminopyralid

LC<sub>50</sub>: 7.08 mg ai/L                      95% C.I.: 5.83-8.61 mg ai/L

**9. ADEQUACY OF THE STUDY:**

**A. Classification:** This study is **scientifically sound** and is classified as **acceptable**.

**B. Rationale:** The study conducted follows the criteria outlined in OPPTS Guideline 850.1035 (Mysid Acute Toxicity Test).

- 10. GUIDELINE DEVIATIONS:** This study was conducted following guidelines outlined in US EPA, OPPTS Ecological Effects Test Guidelines for conducting acute toxicity tests with saltwater mysids (850.1035). The following deviations was noted:
- Dissolved oxygen dropped below 60% of saturation in the aged solution of one replicate of the highest treatment level.

12. **SUBMISSION PURPOSE:** This study was submitted to provide information on the effects of GF-2633 on *Americamysis bahia* survival following acute exposure for the purpose of chemical registration review.

13. **MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species are <i>Mysidopsis bahia</i> , <i>Penaeus setiferus</i> , <i>P. duorarum</i> , <i>P. aztecus</i> and <i>Palaemonetes</i> sp.	<i>Americamysis bahia</i>
<b><u>Age</u></b> Juvenile, mysids should be ≤24 hours old	<24 hours old
<b><u>Supplier</u></b>	Continuous laboratory cultures
<b>All shrimp are from same source?</b>	Yes
<b>All shrimp are from the same year class?</b>	Yes

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b><u>Acclimation Period</u></b> minimum 10 days	N/A; Continuous culture conditions
<b>Wild caught organisms were quarantined for 7 days?</b>	N/A
<b>Were there signs of disease or injury?</b>	Not reported
<b>If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?</b>	N/A

Guideline Criteria	Reported Information
<p><b><u>Feeding</u></b> No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each.</p> <p>Mysids should be fed throughout the study.</p>	Mysids were fed live brine shrimp ( <i>Artemia sp</i> ) nauplii at least once per day during culture and <i>ad libitum</i> during testing.
<p><b><u>Pretest Mortality</u></b> &lt;3% mortality 48 hours prior to testing</p>	Not reported

### C. Test System

Guideline Criteria	Reported Information
<p><b><u>Source of dilution water</u></b> Soft reconstituted water or water from a natural source, <b>not</b> dechlorinated tap water</p>	<p>The saltwater used in the study was prepared by adding commercial sea salt mix to laboratory freshwater. The laboratory freshwater consisted of well water that was demineralized by reverse osmosis. Prior to use, the dilution water was filtered (1 µm) and UV sterilized.</p> <p>The results of the most recent water analysis (February 2011) for selected chemical parameters and potential contaminants was provided.</p>
<b>Does water support test animals without observable signs of stress?</b>	Yes
<p><b><u>Salinity</u></b> 30-34‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range &lt;6‰</p>	19.6-20.0‰
<p><b><u>Water Temperature</u></b> Approx. 22±1°C</p>	24.1-25.3°C

Guideline Criteria	Reported Information
<b><u>pH</u></b> 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range <0.8	7.7-8.2
<b><u>Dissolved Oxygen</u></b> Static: $\geq 60\%$ during 1 <sup>st</sup> 48 hrs and $\geq 40\%$ during 2 <sup>nd</sup> 48 hrs, Flow-through: $\geq 60\%$	<u>New Solutions</u> : 6.8-7.2 mg/L (94-99% saturation) <u>Old Solutions</u> : 3.6-6.8 mg/L (50-94% saturation)  No aeration was added because saturation was <60% in only one replicate of the highest treatment group at test termination.
<b><u>Total Organic Carbon</u></b> Should be <5 mg/L in reconstituted seawater	Not reported
<b><u>Test Aquaria</u></b> 1. <u>Material</u> : Glass or stainless steel 2. <u>Size</u> : 19.6 L is acceptable for organisms $\geq 0.5$ g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. <u>Fill volume</u> : 15 L is acceptable for organisms $\geq 0.5$ g, 2-3 L is acceptable for smaller organisms.	Glass jars with plastic Petri dish cover  500 mL  250 mL fill volume
<b><u>Type of Dilution System</u></b> Must provide reproducible supply of toxicant	N/A; static-renewal system with test solution renewal at 48 hours.
<b><u>Flow Rate</u></b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A

Guideline Criteria	Reported Information
<b><u>Biomass Loading Rate</u></b> Static: < 0.8 g/L at < 17°C, < 0.5 g/L at >17°C; flow-through: ≤ 1 g/L/day (N/A for mysids)	Not reported
<b><u>Photoperiod</u></b> 16 hours light, 8 hours dark	14 hour light:10 hour dark photoperiod with 30-minute transition periods. Light intensity was 746 lux at test initiation.
<b><u>Solvents</u></b> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	N/A

#### D. Test Design

Guideline Criteria	Reported Information
<b><u>Range Finding Test</u></b> If LC <sub>50</sub> >100 mg/L with 30 shrimp, then no definitive test is required.	A static-renewal range-finding test was conducted May 18-22, 2011 at nominal concentrations of 0 (control), 0.10, 1.0, 10, and 100 mg ai/L. The test solutions were renewed at 48 hours. At 96 hours, mortality was 60% in the 100 mg ai/L group. No mortalities were observed in the control or ≤10 mg ai/L groups.
<b><u>Nominal Concentrations of Definitive Test</u></b> Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0 (negative control), 6.3, 13, 25, 50, and 100 mg GF-2633/L.  <u>Aminopyralid triisopropanolammonium</u> 0.53, 1.10, 2.11, 4.22, and 8.43 mg ai/L  <u>2,4-D dimethylamonium</u> 2.71, 5.59, 10.8, 21.5, and 43.0 mg ai/L
<b><u>Number of Test Organisms</u></b> Minimum 20/level, may be divided among containers	20/level (4 replicates/level with 5 mysids/replicate)

Guideline Criteria	Reported Information
<b>Test organisms randomly or impartially assigned to test vessels?</b>	Yes
<b>Biological observations made every 24 hours?</b>	Yes
<b><u>Water Parameter Measurements</u></b> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary >1°C 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temperature, dissolved oxygen, pH, and salinity were measured in all replicate test chambers daily. Temperature was also measured continuously in the waterbath using an electronic data-logging system.
<b><u>Chemical Analysis</u></b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	The “new” test solutions were measured at 0- and 48-hours and the “old” test solutions were measured at 48- and 96-hours using HPLC-UV method.

**14. REPORTED RESULTS:****A. General Results**

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes. Signed and dated Data Confidentiality, Quality Assurance, and GLP compliance statements were provided. The study was conducted in accordance with US EPA GLP standards (40 CFR part 160), with one exception: the most recent analyses of saltwater for potential contaminants (Feb. 2011) was not performed according to the stated GLP.
<b><u>Recovery of Chemical</u></b>	<u>New Solutions</u> : 89 to 104% of nominal <u>Old Solutions</u> : 86 to 106% of nominal
<b><u>Control Mortality</u></b> Not more than 10% of control organisms may die or show abnormal behavior.	0% control mortality
<b>Raw data included?</b>	Yes
<b>Signs of toxicity (if any) were described?</b>	Not reported.

**B. Mortality <sup>a</sup>**

Concentration (mg GF-2633/L)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<MQL <sup>b</sup>	20	0	0	0	0
6.3	5.76	20	0	0	0	1
13	12.5	20	0	1	1	1
25	25.1	20	0	0	0	0
50	51.5	20	0	2	2	2
100	101	20	0	4	10	13*

a Data were obtained from Table 2 on page 21 of the study report.

b MQL= 1.90 mg GF-2633/L

\* Statistically significant mortality compared to the control (Fisher's Exact Test, p=0.05).

**C. Statistical Results**

Statistical Method: Statistical analyses were performed using SAS<sup>®</sup> (version 9.1) statistical software. The LC<sub>50</sub> estimates and 95% confidence limits were calculated using the probit method and Trimmed Spearman-Kärber method. The NOAEC was determined using Fisher's one-tailed exact test. Results are reported using mean measured concentrations.

96 hour mortality:GF-2633

LC<sub>50</sub>: 84.1 mg form/L      95% C.I.: 69.1-102 mg form/L

2,4-D DMA

LC<sub>50</sub>: 36.1 mg ai/L      95% C.I.: 29.7-43.9 mg ai/L

Aminopyralid

LC<sub>50</sub>: 7.08 mg ai/L      95% C.I.: 5.83-8.61 mg ai/L

NOAEC: 51.5 mg GF-2633/L

LOAEC: 101 mg GF-2633/L (based on 96 hour mortality)

**D. Sublethal Effects:** None reported.

## 15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Trimmed Spearman-Kärber Output
GF-2633*	84.1 (69.1-102) mg form/L
2,4-D dimethylammonium	36.1 (29.7-43.9) mg ai/L
Aminopyralid triisopropanolammonium	7.08 (5.83-8.61) mg ai/L
Software	CETIS, Version 1.8.7.12
Backend settings	10/20/15

\*GF-2633 is an adjusted mixture of the proportions of two active ingredients 2,4-D DMA and aminopyralid at the onset of the study.

## 16. REVIEWER'S COMMENTS:

The reviewer's results were comparable to those reported by the study author based on the formulated product. However, the reviewer also expressed toxicity values based on the individual active ingredients. Therefore, the reviewer's results are reported in the Conclusions section of this DER

After 96 hours of exposure, mortality was 0, 5, 5, 0, 10, and 65% in the control, 5.76, 12.5, 25.1, 51.5, and 101 mg GF-2633/L groups, respectively.

GF-2633 is an adjusted mixture based on proportions of 2,4-D DMA and aminopyralid at the onset of the study. The formulated product, GF-2633, consisted of 8.43% wt/wt aminopyralid triisopropanolammonium (i.e, 4.38% wt/wt aminopyralid acid equivalent) and 43.0% wt/wt 2,4-D dimethylammonium (35.7% wt/wt 2,4-D acid equivalent). When calculating the nominal and mean-measured concentrations, the reviewer used the active ingredients (not the acid equivalents).

The in-life phase of the definitive test was performed from June 13-17, 2011.

**17. CONCLUSIONS:**

This study is scientifically sound and classified as acceptable. Based on the results of this study, GF-2633 and 2,4-D DMA would be classified as **slightly toxic** and aminopyralid would be classified as **moderately toxic**.

**18. REFERENCES:**

Rebstock, M. (2011) GF-2633: “Acute Toxicity to the Water Flea, *Daphnia magna*, Determined Under Static Test Conditions”, DAS 110056, ABC Study No. 66953.

All other references were standard guidelines or methodologies.

# CETIS Summary Report

Report Date: 07 Jan-16 05:30 (p 1 of 1)  
 Test Code: 48939503 24D | 20-4827-8524

## OPPTS 850.1035 Acute Invert (Mysid)

ABC Labs

<b>Batch ID:</b>	19-8746-0521	<b>Test Type:</b>	Mortality (96-h)	<b>Analyst:</b>	
<b>Start Date:</b>	11 Jun-13	<b>Protocol:</b>	OPPTS 850.1035 Acute Invert (Mysid Shri	<b>Diluent:</b>	Well Water
<b>Ending Date:</b>	07 Jan-16 05:26	<b>Species:</b>	Americamysis bahia	<b>Brine:</b>	Generic commercial salts
<b>Duration:</b>	940d 5h	<b>Source:</b>	Lab In-House Culture	<b>Age:</b>	<24h

<b>Sample ID:</b>	08-4025-1036	<b>Code:</b>	48939503 24D	<b>Client:</b>	CDM Smith - J. Marton
<b>Sample Date:</b>	11 Jun-13	<b>Material:</b>	2,4-D, dimethylamine salt	<b>Project:</b>	Herbicide
<b>Receive Date:</b>	07 Jan-16 05:26	<b>Source:</b>	Dow AgroSciences		
<b>Sample Age:</b>	NA	<b>Station:</b>			

**Batch Note:** PC Code 030019+005100, MRID 48939503, mean-measured 2,4D-DMA concentrations

**Sample Note:** PC Code 030019+005100, MRID 48939503, mean-measured 2,4D-DMA concentrations

### Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
17-1538-2708	96h Mortality Rate	LC5	6.95	5.46E-07	15		Linear Regression (MLE)
		LC10	10.6	0.000438	21.8		
		LC15	14.2	0.0336	33.2		
		LC20	17.8	0.716	68.8		
		LC25	21.7	4.26	298		
		LC40	35.5	17.1	267000		
		LC50	47.7	23.1	27100000		
13-6111-1819	96h Mortality Rate	LC50	36.1	29.7	43.9		Trimmed Spearman-Kärber

### 96h Mortality Rate Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0	0	0	0	0	0	0		
2.48		4	0.05	0	0.209	0	0.2	0.05	0.1	200.0%	
5.38		4	0.05	0	0.209	0	0.2	0.05	0.1	200.0%	
10.8		4	0	0	0	0	0	0	0		
22.1		4	0.1	0	0.418	0	0.4	0.1	0.2	200.0%	
43.4		4	0.65	0.25	1	0.4	1	0.126	0.252	38.7%	

### 96h Mortality Rate Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0	0	0	0
2.48		0.2	0	0	0
5.38		0.2	0	0	0
10.8		0	0	0	0
22.1		0	0	0	0.4
43.4		0.4	1	0.6	0.6

# CETIS Summary Report

Report Date: 07 Jan-16 05:43 (p 1 of 1)  
 Test Code: 48939503 amino | 08-1233-8155

## OPPTS 850.1035 Acute Invert (Mysid)

ABC Labs

<b>Batch ID:</b>	04-7494-3690	<b>Test Type:</b>	Mortality (96-h)	<b>Analyst:</b>	
<b>Start Date:</b>	11 Jun-13	<b>Protocol:</b>	OPPTS 850.1035 Acute Invert (Mysid Shri	<b>Diluent:</b>	Well Water
<b>Ending Date:</b>	07 Jan-16 05:30	<b>Species:</b>	Americamysis bahia	<b>Brine:</b>	Generic commercial salts
<b>Duration:</b>	940d 5h	<b>Source:</b>	Lab In-House Culture	<b>Age:</b>	<24h

<b>Sample ID:</b>	04-1852-0216	<b>Code:</b>	48939503 amino	<b>Client:</b>	CDM Smith - J. Marton
<b>Sample Date:</b>	11 Jun-13	<b>Material:</b>	Aminopyralid	<b>Project:</b>	Herbicide
<b>Receive Date:</b>	07 Jan-16 05:30	<b>Source:</b>	Dow AgroSciences		
<b>Sample Age:</b>	NA	<b>Station:</b>			

**Batch Note:** PC Code 030019+005100, MRID 48939503, mean-measured aminopyralid concentrations

**Sample Note:** PC Code 030019+005100, MRID 48939503, mean-measured aminopyralid concentrations

### Point Estimate Summary

Analysis ID	Endpoint	Level	lbs ai/A	95% LCL	95% UCL	TU	Method
04-8616-9191	96h Mortality Rate	LC5	1.36	3.47E-07	2.93		Linear Regression (MLE)
		LC10	2.09	0.000174	4.26		
		LC15	2.78	0.00981	6.47		
		LC20	3.49	0.166	13.1		
		LC25	4.25	0.876	51.6		
		LC40	6.96	3.36	28000		
		LC50	9.36	4.55	2060000		
00-9226-7165	96h Mortality Rate	LC50	7.08	5.83	8.61		Trimmed Spearman-Kärber

### 96h Mortality Rate Summary

C-lbs ai/A	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0	0	0	0	0	0	0		
0.49		4	0.05	0	0.209	0	0.2	0.05	0.1	200.0%	
1.05		4	0.05	0	0.209	0	0.2	0.05	0.1	200.0%	
2.12		4	0	0	0	0	0	0	0		
4.34		4	0.1	0	0.418	0	0.4	0.1	0.2	200.0%	
8.51		4	0.65	0.25	1	0.4	1	0.126	0.252	38.7%	

### 96h Mortality Rate Detail

C-lbs ai/A	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0	0	0	0
0.49		0.2	0	0	0
1.05		0.2	0	0	0
2.12		0	0	0	0
4.34		0	0	0	0.4
8.51		0.4	1	0.6	0.6

# CETIS Summary Report

Report Date: 07 Jan-16 05:41 (p 1 of 1)  
 Test Code: 48939503 form | 02-1062-7270

## OPPTS 850.1035 Acute Invert (Mysid)

ABC Labs

<b>Batch ID:</b>	14-5651-1564	<b>Test Type:</b>	Mortality (96-h)	<b>Analyst:</b>	
<b>Start Date:</b>	13 Jun-11	<b>Protocol:</b>	OPPTS 850.1035 Acute Invert (Mysid Shri	<b>Diluent:</b>	Well Water
<b>Ending Date:</b>		<b>Species:</b>	Americamysis bahia	<b>Brine:</b>	Generic commercial salts
<b>Duration:</b>	NA	<b>Source:</b>	Lab In-House Culture	<b>Age:</b>	<24h

<b>Sample ID:</b>	13-6093-0335	<b>Code:</b>	48939503 form	<b>Client:</b>	CDM Smith - J. Marton
<b>Sample Date:</b>	13 Jun-11	<b>Material:</b>	2,4-D DMA + Aminopyralid	<b>Project:</b>	Herbicide
<b>Receive Date:</b>		<b>Source:</b>	Dow AgroSciences		
<b>Sample Age:</b>	NA	<b>Station:</b>			

**Batch Note:** PC Code 030016+005100, MRID 48939503, mean-measured formulation concentrations

**Sample Note:** PC Code 030016+005100, MRID 48939503, mean-measured formulation concentrations

### Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
16-8416-6197	96h Mortality Rate	LC5	16.2	1.33E-06	34.8		Linear Regression (MLE)
		LC10	24.7	0.00105	50.7		
		LC15	33	0.0795	77.3		
		LC20	41.4	1.68	160		
		LC25	50.4	9.94	691		
		LC40	82.6	39.7	608000		
		LC50	111	53.8	61000000		
00-1241-9290	96h Mortality Rate	LC50	84.1	69.1	102		Trimmed Spearman-Kärber

### 96h Mortality Rate Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0	0	0	0	0	0	0		
5.76		4	0.05	0	0.209	0	0.2	0.05	0.1	200.0%	
12.5		4	0.05	0	0.209	0	0.2	0.05	0.1	200.0%	
25.1		4	0	0	0	0	0	0	0		
51.5		4	0.1	0	0.418	0	0.4	0.1	0.2	200.0%	
101		4	0.65	0.25	1	0.4	1	0.126	0.252	38.7%	

### 96h Mortality Rate Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0	0	0	0
5.76		0.2	0	0	0
12.5		0.2	0	0	0
25.1		0	0	0	0
51.5		0	0	0	0.4
101		0.4	1	0.6	0.6